Processes Affecting the Variability of Fluorescence Signals from Benthic Targets in Shallow Waters

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LONG-TERM GOAL

Our major theme is to understand and to qualify processes that contribute to fluorescence emission from benthic targets in the coastal and shallow waters with the overarching goal of developing parametrization schemes that optically detect anthropogenic objects.

SCIENTIFIC OBJECTIVE:

1. To identify the sources of variations in the effective absorption cross sections of target molecules and of fluorescence lifetimes (and by inference, quantum yields) of individual chromophores, and to provide an interpretive understanding of how physical, chemical, and biological variability affects these optical properties.

2. To determine the extent and variability in the coupling of absorbed radiation to the fluorescence emission spectrum, and the development of biophysical radiative transfer models that predict the latter from the former in a variety of benthic environments.

3. To develop an understanding of the spatial and temporal variability in benthic and optical signals.

APPROACH

Our basic approach to study the variability in fluorescence signals is to use the Fast Repetition Rate (FRR) Fluorometry technique (Falkowski P.G. and Kolber Z. 1995; Kolber et al, 1998). The FRR

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technique measures fluorescence transients induced by a sequence of subsaturating flashes, where the intensity, duration, and interval between them is precisely controlled. A comprehensive suite of fluorescence yields and photosynthetic parameters is calculated from the fluorescence transients.

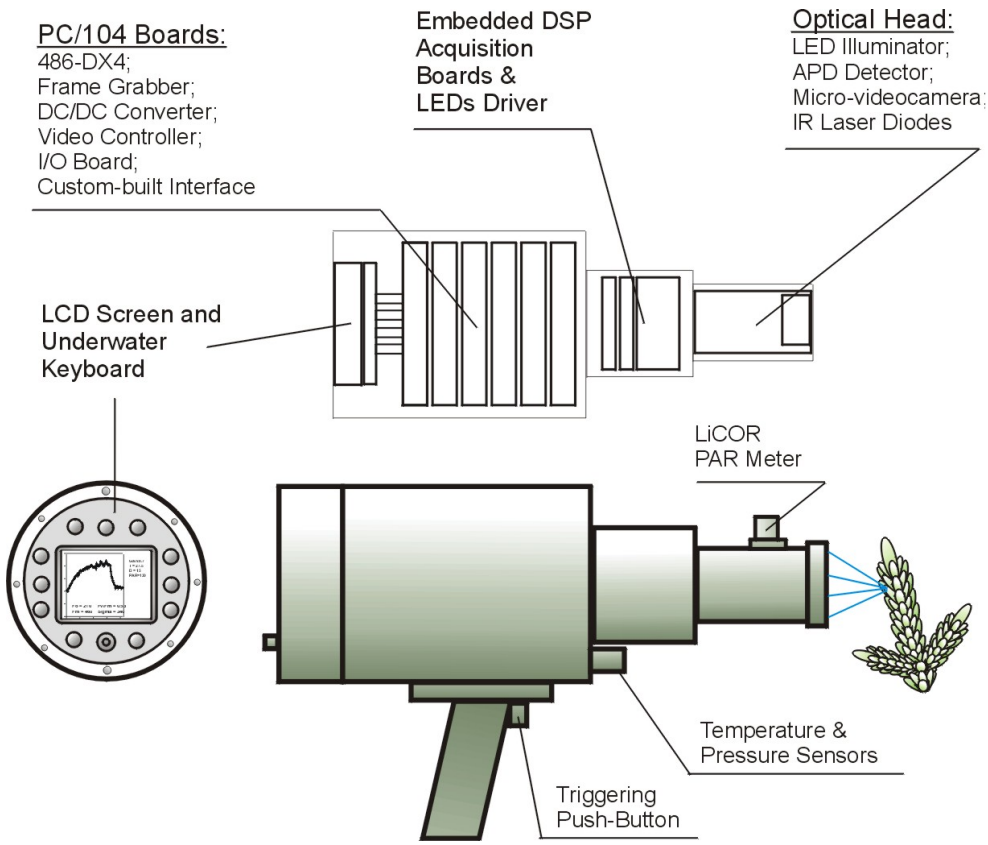

Figure 1. Scheme of the SCUBA-based Fast Repetition Rate (FRR) Fluorometer. Fluorescence is excited by a sequence of flashes generated by a bank of ultra-bright blue LEDs (excitation wavelength 460 nm, FWHM 20 nm). The induced fluorescence transients are recorded by an avalanche photodiode (APD) module. The excitation protocols and data acquisition are controlled by an embedded Digital Signal Processing circuit interfaced to a PC/104 486DX4 computer board. A compact video camera is incorporated into the instrument, allowing the diver to monitor the target in real-time and to capture the image simultaneously with the fluorescence measurements. A pair of orthogonal, off-axis IR laser diodes is incorporated in the viewfinder to control the distance to the target. Both fluorescence data and images are stored in the on-board Flash Memory Card and downloaded after a dive.

Within the frame of CoBOP, we designed and constructed a SCUBA-based FRR fluorometer (Fig. 1 and Gorbunov et al., 1999), which allows the fluorescence parameters in benthic targets to be measured instantaneously, *in situ*, and with high spacial and temporal resolution. Our first observation of temporal variability in corals and seagrasses, conducted in May 1998, showed that fluorescence yields can exhibit very high diel variations, reaching 3 to 4-fold, depending on irradiance and photosynthetic state. These measurements indicated that prolonged operation of a moored instrument without diver attention was required for quantifying, in detail, the temporal variability *in situ*. To achieve this goal, we developed the 3rd version of the SCUBA-based FRR Fluorometer (Fig.2). First, we modified the

optical scheme of the instrument and increased the optimal operational distance between the instrument and the object, thereby eliminating any disturbance of the object during long-term observations. Second, we designed and built a new corrosion-free underwater housing suitable for prolonged underwater operation. Finally, we constructed an appropriate benthic platform for mooring the instrument for a long period of time. A large capacity (15 Ah) battery pack, with an incorporated timer, provided autonomous data acquisition for 2-3 days during field measurements on remote sites. Alternatively, for long-term (e.g. seasonal) observations on a near-shore coral reef, the instrument was powered by a laboratory power supply through a 300 feet waterproof cable. The cable was also used for downloading data into a laptop computer, installed in the laboratory.

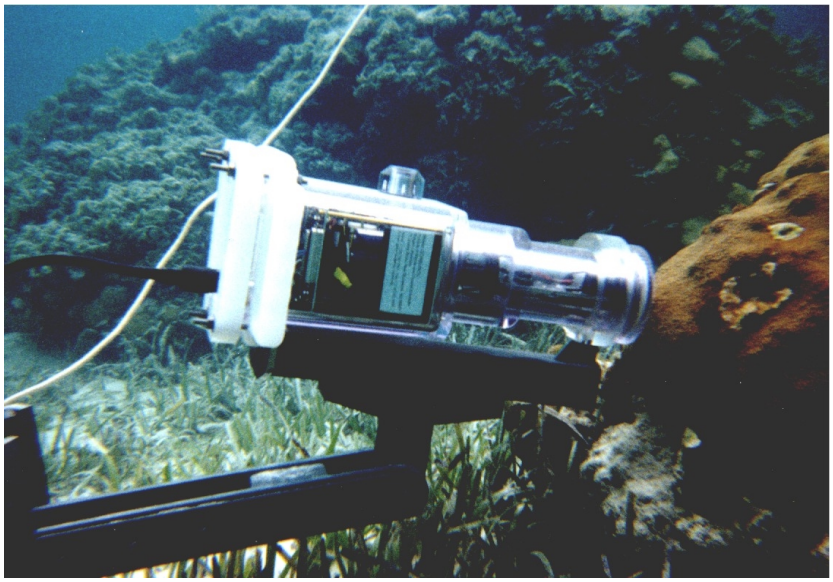

Figure 2. 3rd version of the SCUBA-based FRR Fluorometer installed on a benthic platform next to a coral head at Lee Stocking Island.

WORK COMPLETED

Two SCUBA-based FRR fluorometers were employed during the CoBOP-99 field campaigns (in January and May 1999) at Lee Stocking Island and were used to study a wide variety of benthic targets. Using the diver-operated instrument, over one thousand measurements were made on corals, surficial sediments, seagrasses, and macroalgae in both *in situ* and in lab aquaria. These measurements were analyzed in conjunction with the data collected previously to identify and quantify unique fluorescent and photosynthetic signatures of benthic targets. The new moorable SCUBA-based FRR fluorometer was deployed on a benthic platform (Fig.2) to record diel cycles of fluorescence signals from selected targets. 60 diel cycles have been acquired *in situ* on various benthic organisms under different light regimes and 35 cycles - in lab aquaria. From these measurements, we determined and quantified the mechanisms responsible for the diel variability in fluorescence from benthic organisms. A biophysical model is being developed to describe temporal variability in fluorescence yields. Relying on this model, we expect to provide algorithms that predict the patterns of diel variability in fluorescence. Initial data analysis has been completed and files for the database were prepared.

In 1999, we continued to study the origin and variability of orange and green fluorescence from corals (this work is conducted in collaboration with Dr. C.Mazel and M.Lesser). To more precisely determine the identity of the fluorophore(s), we isolated green fluorescent proteins from a variety of cnidarians and characterized their spectral signatures and biochemical characteristics.

RESULTS

Temporal variability of fluorescence yields in benthic targets.

In 1999, our particular emphasis was given to the study of temporal variability of fluorescence signals from benthic organisms. Fig. 3 shows an example of diel variations in fluorescence yields and photosynthetic parameters measured *in situ* with the new moored FRR instrument. Fig. 4 presents temporal variability in fluorescence recorded during a month at LSI.

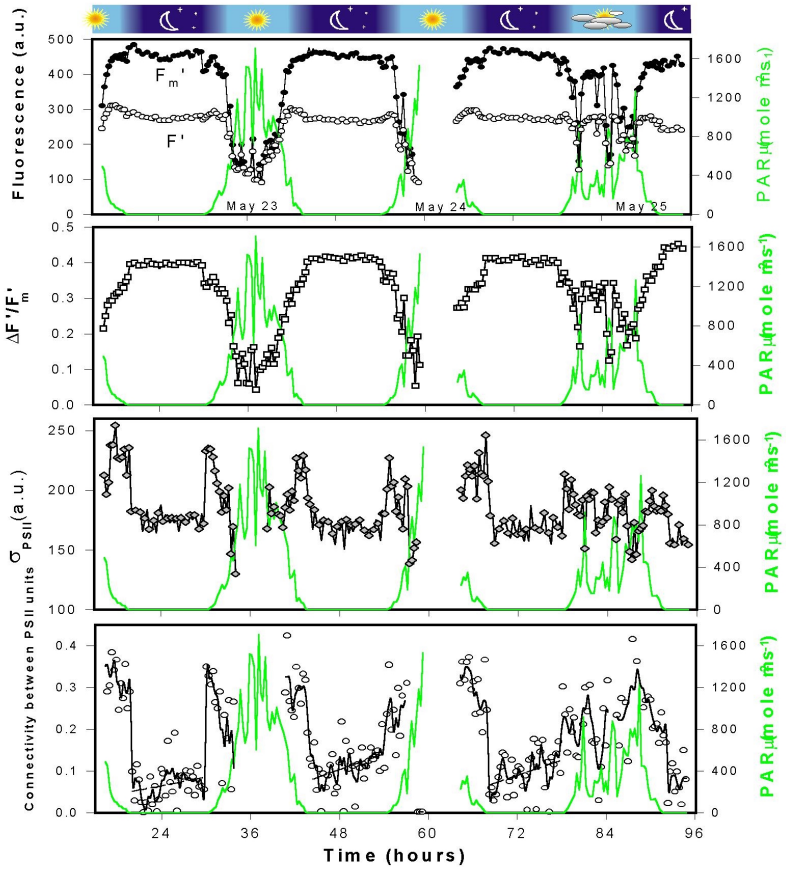

Figure 3. Diel cycles of fluorescence yields and photosynthetic parameters measured in situ on a coral head of *Montastraea faveolata* ***in shallow waters (2 m depth). (a) - variations in chlorophyll fluorescence at steady-state (F=) and maximum (F_m=) levels, (b) - quantum yield of photochemistry in PSII ($\Delta F=/F_m=$), (c) - functional absorption cross section for PSII (σ_{PSII}), and (d) - energy transfer between PSII units (Aconnectivity factor≅).***

The main results from the study of diel variability of fluorescence yields are as follows:

(1) Chlorophyll fluorescence can vary up to 3-4 fold during a day depending on organisms, photosynthetic activity and irradiance.

(2) Diel variations in fluorescence are driven by indirect irradiant variations in the quantum yield of fluorescence.

(3) The major mechanism of diel variability in the quantum yield of fluorescence from corals is non-photochemical quenching of chlorophyll fluorescence (explains ~80% of variability).

(4) Non-photochemical quenching is associated with non-radiative deactivation of excited states in the pigment bed and accompanied with a decrease in the functional absorption cross section of PSII (σ_{PSII}).

(5) Non-photochemical quenching is driven by the rate of photosynthetic electron transport through PSII. As a result, the coefficient of non-photochemical quenching is proportional to the extent of saturation of electron transport caused by ambient light.

(6) Non-photochemical quenching depends on the organism, its photosynthetic performance and environmental factors:
- it is less pronounced in benthic targets with high σ_{PSII};
- it is higher in stressed (e.g. nutrient limited) organisms;
- it is higher, at the same level of irradiance, in organisms acclimated to low light.

(7) Photoinhibition of photosynthesis is small in natural benthic communities and explains $< 20\%$ of the diel variability in fluorescence.

(8) Patterns of diel variability can be assessed and predicted from knowledge of irradiance and photosynthetic parameters, measured with the FRRf.

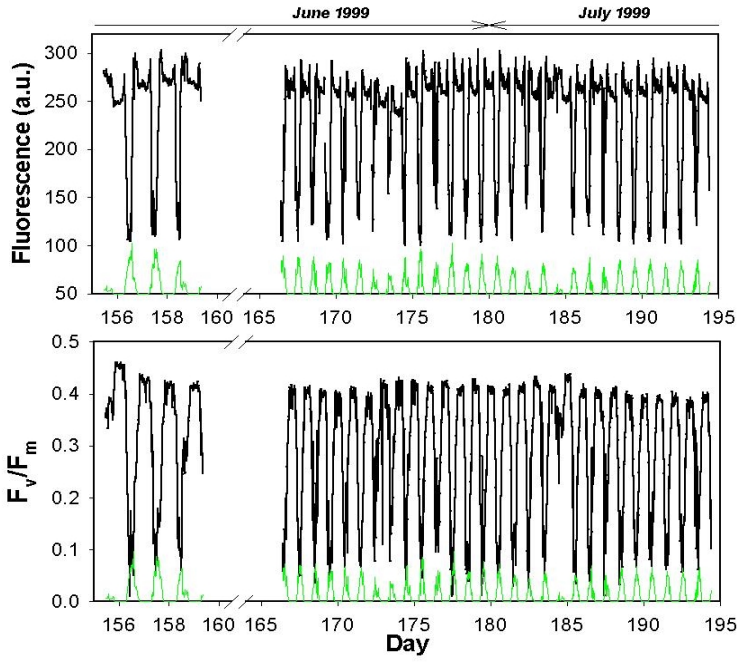

Figure 4. Temporal variability in fluorescence and quantum yield of on-going photochemistry in PSII (F_v/F_m), measured for a month in the coral M. faveolata in shallow waters (2 m depth) at Lee Stocking Island. Grey lines are in situ irradiance. The fluorescence yield was maximal at night and declined up to 2-3 fold during a day due to non-photochemical quenching of chlorophyll fluorescence. The non-photochemical quenching is directly governed by the extent of saturation in the photosynthetic electron transport through PSII, resulting in an irradiance-induced reduction in F_v/F_m.

FRR fluorescence signatures of benthic targets.

The FRR fluorometry allowed for specific optical signatures of benthic targets to be identified and quantified.

A summary on the fluorescence and photosynthetic signatures of benthic targets:

(1) Different benthic organisms exhibit unique FRR fluorescence and photosynthetic signatures that can be employed for identification of various benthic targets by the FRR techniques.

(2) Corals have low photosynthetic efficiency (F_v/F_m averages 0.40) and moderate functional absorption cross sections (σ_{PSII}).

(3) Algal turfs have the highest σ_{PSII} and high photosynthetic efficiency (F_v/F_m averages 0.50).

(4) Seagrasses exhibit low σ_{PSII} and very high photosynthetic efficiency (F_v/F_m 0.70-0.73).

(5) Macroalgae are characterized by the highest level of variability in fluorescence yields and photosynthetic efficiency, attributed to environmental variations in the nutrient status of these benthic organisms.

Origin and properties of green and orange fluorescence from corals.

In collaboration with Drs. C. Mazel and M.Lesser, we continue to study both green and orange fluorescence in corals. The main results on the properties of the green fluorescence are as follows:
(1) There is a striking similarity between the spectral signatures of coral green fluorophores and GFPs isolated from bioluminescent coelenterates *Aequorea* and *Renilla* (Ward, 1979).

(2) Spectral characterization of GFPs isolated from selected corals reveals a sharp emission band with a maximum at ca. 510-515 nm and a shoulder at 540 nm, which was identical for all corals (Fig.5). The pattern of the fluorescence excitation spectra, however, varied essentially with species, implying that two distinctive types of GFP-like fluorophores are present in Cnidarians. The first one had the only excitation band centered at ca. 495 nm; the second type had two absorption bands, one centered at ca. 410 nm and a second at ca. 490 nm (Fig 5).

(3) Fluorescence lifetime analysis with picosecond time resolution suggests a single singlet emission with a lifetime of 2.85 ns, similar to that of GFPs from bioluminescent coelenterates (ranging from 2.8 ns to 3.2 ns (Ward et al., 1980; Swaminathan et al., 1997; Kneen et al., 1998).

(4) Based on the measured fluorescence lifetime, the spectral parameters and the approximate molar extinction coefficient of 42,000 M^{-1} cm^{-1} (Ward, 1979; Swaminathan et al., 1997), we estimate the quantum yield of coral GFP fluorescence to be about 35%.

(5) Biochemical characterization of coral GFPs suggests that the smallest monomeric form of the protein runs with an apparent molecular mass of ca. 30 +/-1 kDa on denaturing SDS-PAGE. Under non-denaturing conditions however, the monomeric protein elutes at 27 +/- 1 kDa in a well calibrated size-exclusion column. In some species the non-denatured protein elutes as a tetramer and/or dimer. None of the coral GFPs isolated by SDS-PAGE cross reacts with polyclonal antibodies raised against either GFP from the jelly-fish (*Aequorea*) or the Sea Pansy (*Renilla*).

(6) Epifluorescence microscopy suggests that the green fluorescence is localized in amorphous intracellular bodies that range in size from ca. 1 to 5 um.

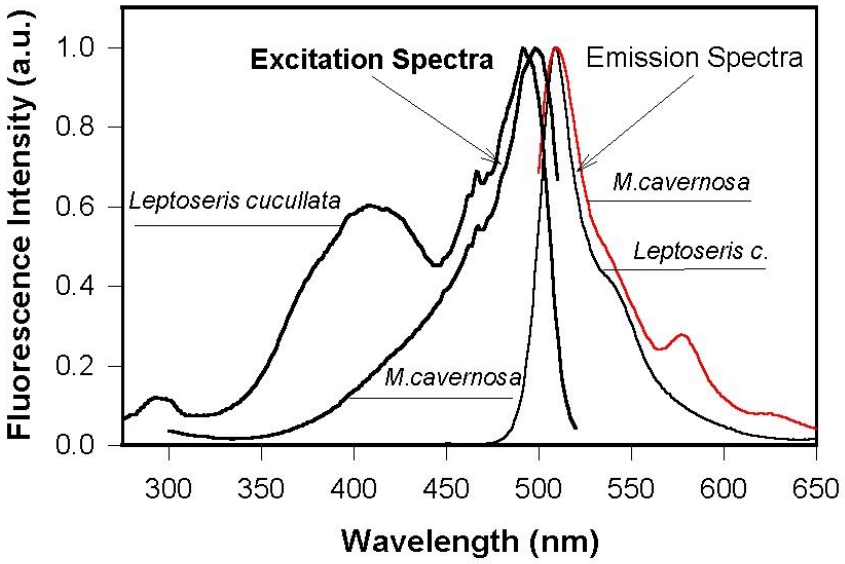

Figure 5. Fluorescence emission and excitation spectra of the green fluorescent proteins (GFPs) from the corals *Leptoseris cucculata* ***and*** *Montasraea cavernosa.* ***Both GFPs have identical emission spectra, but different excitation spectra.***

During the CoBOP field studies at LSI in May 1999, we observed a novel orange chromophore (called below PE-608), which is responsible for a strong emission band in the orange-red spectral region in selected coral species, and characterized its spectral properties. This pigment was found in the reef-building corals *Porites astreoides* (green variety), populating benthic reef communities in shallow waters. Presence of this chromophore substantially modified the color and spectral reflectance of the coral, as compared to grey variety of *Porites astreoides*, lacking the orange pigment. To our knowledge, this chromophore has not been documented previously in corals or any other photosynthetic organisms. The PE-608 excitation spectrum is identical to that of phycoerythrins from a variety of coral species explored previously. The emission spectrum is, however, shifted ca. 30 nm to the red region and has a different pattern. Based on its spectral properties, we attribute PE-608 to a family of phycoerythrins, photosynthetic pigments occurring in cyanobacteria, chriptomonads and red algae. The spectral patterns of PE-608 are similar to that of Phycoerythrin 566, where fluorescence emission is shifted much further into the red than that of other phycoerythrins and which has been found only in selected species of chriptomonads. Epifluorescent microscopic analysis indicated that PE-608 is localized in unicellular cells living symbiotically in coral tissue. We imply that the orange fluorescence originates from endosymbiotic chriptomonads living in animal host of the corals. Further optical and biochemical analysis is required to identify this novel chromophore and determine its physiological function.

IMPACT/APPLICATON

Understanding the sources of variability and behavior of benthic fluorescent targets, such as corals, turf algae, invertebrates, seagrasses, and macroalgae is essential to developing operational protocols for distinguishing between anthropogenic and naturally occurring objects. Moreover, within the overall goals of CoBOP, namely identification and quantitation of IOPs that are required for closure of radiative transfer models, fluorescence is a source of spectrally camouflaged photons that are radiated from benthic targets that have absorbed photons at other wavelengths. Thus, the consideration of varying fluorescence processes, in conjunction with the measurements of spectral absorption and scattering, will lead to numerically accurate and complete radiative transfer models. In addition, the set of fluorescent and photosynthetic signatures of benthic targets acquired by the SCUFRR fluorometer can be applied to the interpretation of fluorescence images obtained with the Laser Line Scanner, as well as for corrections to measure reflectance in the red spectral region.

TRANSITIONS

Dr. Michael Lesser (Univ. of New Hampshire) has been using the SCUBA-based FRR fluorometers in an ONR-funded project, on the effect of spectral quality and quantity in the underwater light field and elevated temperatures on small scale optical properties of corals, and in a NOAA-funded study of coral bleaching phenomena. The FRR fluorescence data on corals and sediments are used by Drs. Fred Dobbs, Jules Jaffe and Dave Zawada within the frame of CoBOP. One of the SCUBA-based FRR instruments is also used by Professor Zvy Dubinsky and his colleagues at Bar-Ilan University (Ramat Gan, Israel) for exploration of coral reefs in the Red Sea.

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